



Shade or Light?: Size Class Distribution and $\delta^{13}\text{C}$ values
as clues to the recruiting environment of *Widdringtonia*
whytei

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Abstract

In order to determine the recruitment environment of the endangered Mulanje Cedar, *Widdringtonia whytei*, size class distributions were measured for six populations on Mount Mulanje, Malawi. Carbon isotope ratios were also collected from leaf tissue in trees of varying height in order to determine the effects of positioning within or below the canopy. Carbon isotope ratios were also taken from wood cores and the $\delta^{13}\text{C}$ values for the innermost and outermost wood were compared for two sites. Size class frequency distributions had a tendency to be normally distributed but one site showed skewed values towards seedling and another showed a flat distribution, possibly due to continual logging of the trees. Height correlated well with leaf $\delta^{13}\text{C}$ values. Sombani and Bvunje showed the same current (outer wood) $\delta^{13}\text{C}$ values but different recruitment (inner wood) $\delta^{13}\text{C}$ values. Results indicate that Bvunje trees recruited in the shade and Sombani recruited in the sunlight. There is ambiguity in both size class data and carbon isotope data and therefore either *Widdringtonia whytei* is able to recruit in a variety of manners and environments or other factors are influencing the results.

Introduction

The Mulanje cedar, *Widdringtonia whytei*, is often confused with *Widdringtonia nodiflora*. However *W. whytei* is a distinctly different species to *W. nodiflora* (Pauw and Linder 1997). *W. nodiflora* is a resprouter commonly found in either grassland or as a component of fynbos, both of which are fire prone vegetation types. The tall mulanje cedar, however, is endemic to the Mulanje massif in Malawi, while the shorter, multi-stemmed, shrub-like *W. nodiflora* occurs all the way from Mount Mulanje to Cape Town in South Africa (Pauw and Linder 1997). Usually occurring between 1500m and 2200 m.a.s.l, *W. whytei* attains a height of between 30-40m with a trunk diameter of around 1m (Chapman 1995; Bayliss *et al* 2007). Cedars have a thick spongy bark and leaves which change with to growth stage; light green, spiky and spirally arranged in juveniles, with the mature trees having leaves that are darker in colour and lie close to the stem (Chapman 1995).

Mulanje cedars are now listed by the IUCN as threatened (Bayliss *et al* 2007). The primary reasons for the red data listing are anthropogenic overexploitation and anthropogenically induced fire. Anthropogenic fires are typically caused by uncontrolled domestic fires or those

ignited in order to flush prey out of the vegetation (Bayliss *et al* 2007) . Mulanje cedars produce a very valuable, durable wood and have consequently been extensively logged (Chapman 1995). The dry woody material leftover from past as well as on-going logging exacerbate the fires amongst cedar stands. The introduced mite, *Cinara cupressi*, is also considered a threat to the Mulanje cedar and the degree to which is controlled by the parasitic wasp, *Pausia bicolour*, has yet to be established (Bayliss *et al* 2007).

Although it has been suggested that frequent fire and anthropogenic overexploitation are primary reasons for the decline of the species, its ecology, particularly its recruitment, is still poorly understood. There is very little evidence to detail the exact relationship between fire and the Mulanje cedar and its vulnerability or reliance on fires (Warren *et al* 2001 cites EDG 2000). However, it has been suggested that Mulanje cedars are shade intolerant requiring disturbance events such as fires to recruit (Chapman 1995). According to Chapman(1995) cedars establish after catastrophic disturbance such as fire, which is reflected in its failure to regenerate beneath the established stand. However, it is also said to require fire to open up gaps for regeneration as it is unable to do so under the closed canopy. Landslides are also a potential form of disturbance allowing for cedar recruitment (Chapman 1995).

Numerous studies show the Mulanje cedar to have high mortality rates as well as low regeneration rates (Bayliss *et al* 2007). By comparing their results with previous studies, Bayliss *et al* (2007) estimated the population decline of Mulanje cedars to be 2.8% per year. They calculated the current area of standing cedars to be 845.3ha. On average stands show 32.6% of standing trees are dead, a result which agrees with previous research (Bayliss *et al* 2007). The rapid loss of the Mulanje cedar, coupled with evidently low regeneration rates places a large amount of stress on the remaining populations.

In order to understand how these trees can be better protected we need to investigate the patterns of regeneration among the Mulanje cedar and whether or not they recruit in shade. Several studies (e.g. Chevillat *et al* 2005; West *et al* 2001; Berry *et al* 1997) have shown a significant difference between stable carbon isotope ratios in leaves growing in full sunlight above the canopy and those growing in the understory. Those lower down in the canopy tend to have more negative $\delta^{13}\text{C}$ values.

The relative proportion of ^{12}C to ^{13}C in the atmosphere is 98.9 to 1.1 (Farquhar *et al* 1989). This ratio does change with photosynthesis as the enzyme RuBP carboxylase discriminates against the heavier ^{13}C isotope when assimilating CO_2 (Farquhar *et al* 1989; February and Stock 1999; Warren *et al* 2001).

CO_2 can become depleted in ^{13}C , close to the forest floor, in closed canopy forests, such as those of Mount Mulaje (Sternberg *et al* 1989). This is because the isotope ratio of the source CO_2 influences the that of the leaf and bacterial decomposition of leaf litter favours the heavier carbon isotope. This results in a distinct difference in isotope ratio between the air on the forest floor and well mixed by air higher up in the canopy (Sternberg *et al* 1989).

Fractionation and the relationship between carbon isotope values and environmental conditions makes it possible to infer the conditions under which cedars recruited. West *et al* (2001) found that it is possible to predict the recruitment environment of trees by comparing $\delta^{13}\text{C}$ values of the inner and outer wood of the tree. However, Chevillat *et al* (2005) points out that there is great variability in carbon isotope signatures both within and between species. In testing whether or not West *et al* (2001)'s findings are applicable to *Widdringtonia whytei* I am able to calibrate my results relative to the $\delta^{13}\text{C}$ values of the leaves of different size class of trees for the same sites.

If Mulanje cedars only recruit after disturbance, as Chapman (1995) suggests, then all trees in a stand would recruit simultaneously as a single cohort, during which time they would be exposed to full sunlight. Here, I firstly examine the size class structure of six of clusters of cedars to determine whether the trees recruited simultaneously. I then use the stable carbon isotope ratios of wood from the centre and periphery of the trunk to establish whether or not recruitment was in the shade.

Study Site:

Mount Mulanje is a large granite inselberg which lies in the southern region of Malawi at 16°S 36°E. Some its highest peaks attain an altitude of more than 3000m but the plateau is closer to 1800m.a.s.l (Beard 1997). The batholith covers an area of around 680 km². The area receives around 2000-3000 mm annual rainfall with miombo woodland on the lower slopes and, grassland and afro-montane forest on the plateau (Beard 1997). The Mulanje cedars are canopy emergents (Pauw and Linder 1997).

The cedar populations visited on the mountain include Sombani (S 15°53'31.0" E 35°41'3.0"), Nkhata (S15°52'49.6" E 35°42'0.3"), Chinzama (S15°53'53.0" E35°39'15.9"), Bvunje (S 15°55'31.2" E 35°35'56.3), Lechenya A (S 15°58'0.36" E 35°33'12.5") and Lechenya B (S 15°58'22.1 E35°33'0.28"). Within each population cedars are typically canopy emergents with an often dense understory of broad leaved angiosperms including *Maesa lanceolata*, *Podocarpus latifolius* and *Apholia theiformis*. Very little light penetrates through to the forest floor in undisturbed populations.

Sombani is a healthy population with a closed canopy, moderately dense undergrowth and no signs of recent disturbance. The Nataka site is angled on a steep slope with particularly dense vine-like undergrowth after a recent fire, possibly January 2010, five months before sampling. Several trees were burnt and/or dead, resulting in an open canopy. Chinzama population was growing among large granite blocks on the edge of a stream, with very old mature, thick undergrowth and a few gaps in and otherwise closed canopy.

Bvunje is a severely anthropogenically disturbed population. There were signs of both past and very recent logging with wood chips strewn on the ground. Many of the trees which had been removed were very large and old. A fire had also killed several trees and many of the non-cedars, such as *Podocarpus latifolius*, were showing signs of overexposure to sunlight due to the sudden opening of the canopy. New weedy growth was covering the ground in the bottom section of the population.



Figure A: Google Earth image of Mount Mulanje, showing all the sites visited.

Lichenya A had a section where fire had opened the canopy, and a section with much denser closed canopy. The understory tended to be denser where the canopy was more open and less dense in parts where it was completely closed. Lichenya B is a closed canopy forest growing on the banks leading down to a river. The undergrowth is dense and moist. Lichenya A and Lichenya B sites were very close together and lay on opposite sides of the Lichenya river.

Methods:

Size Class Distributions

To determine the size class distribution of the cedars trees on Mount Mulanje I measured the circumference at breast height of as many trees as possible in all six populations visited.

Stable Carbon Isotope Analysis

From the Sombani population cores were removed from 10 trees using a increment borer with a 3mm internal diameter. At Bvunje a number of trees had been recently logged. I took samples of the innermost and outmost wood from 10 trees using a sharp knife, from the same sites. At the same sites cores were also removed from 11 non cedar trees. These species include *Kiggelaria Africana*, *Rhamnus prinoides*, *Podocarpus latifolius* and *Ilex mitis*. From the Chinzama and Bvunje 10 leaf samples were also taken from the same non-cedar trees which were cored. Leaf samples were also taken from 29 cedars at Bvunje, Lechenya A and Lechenya B varying in height and life history stage from seedling, sapling and those trees emerging from the canopy. Circumference was measured for these trees using a tape measure and height was estimated. In the case of seedlings, height was measured with a tape measure and circumference was estimated, as these trees were too narrow to measure with a measuring tape. The leaf samples for the non cedars were oven dried overnight at 80°C before being ground to a fine powder using a Retsch MM200 ball bearing mill.

The more mobile resins waxes and oils were removed from the cedar wood and leaf samples using the extraction methods described by Leavitt and Danzer (1993). Finely cut up sections of wood and leaves were placed in pouches constructed from Ankom F57(New York, USA) glass fibre filter bags with a pore size of 25microns and boiled in deionised water for 14 hours. The pouches were then placed in a Soxhlet extractor for 17hrs in 400ml of 1:1 toluene-ethanol mix. The pouches were then dried at 40°C until dried. The Soxhlet extraction process was repeated with pure ethanol for a further 17 hours. The pouches were re-dried and boiled in Erlenmeyer flask with 1000ml deionised water. This final boiling may also removes several inorganic salts, gums and polysaccharides (ref). The samples were then dried again overnight at 40°C.

Samples were then ground by rapid oscillation in Eppendorf tubes using a Retsch MM ball bearing mill (Retsch Haan Germany). Two milligrams of each sample was analysed in a Finnigan Matt 252 mass spectrometer (UCT). $\delta^{13}\text{C}$ values were calculated using the equation:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000$$

Carbon isotope ratios were corrected for by adding 2‰ to the outer part of the wood to correct for post- industrial shift in $\delta^{13}\text{C}$ values in atmospheric CO_2 . The resultant $\delta^{13}\text{C}$ values for the centre of the tree were estimated to be older than fifty years and were therefore corrected by 2‰ for the fossil fuel contribution to atmospheric $\delta^{13}\text{C}$ values (feruary and stock 1999). Graphs were plotted in Windows Excel 2007 and Wilcoxon matched pairs test and Mann Whitney U tests were performed in Statistica 9.0.

Results

Size class distributions

The size class frequencies of both Sombani and Nataka populations are approaching normal distributions, but lack juveniles in the smallest (0-19cm) size class. Chinzama is heavily skewed to the right, with high numbers of juveniles in the 0-19cm size class.

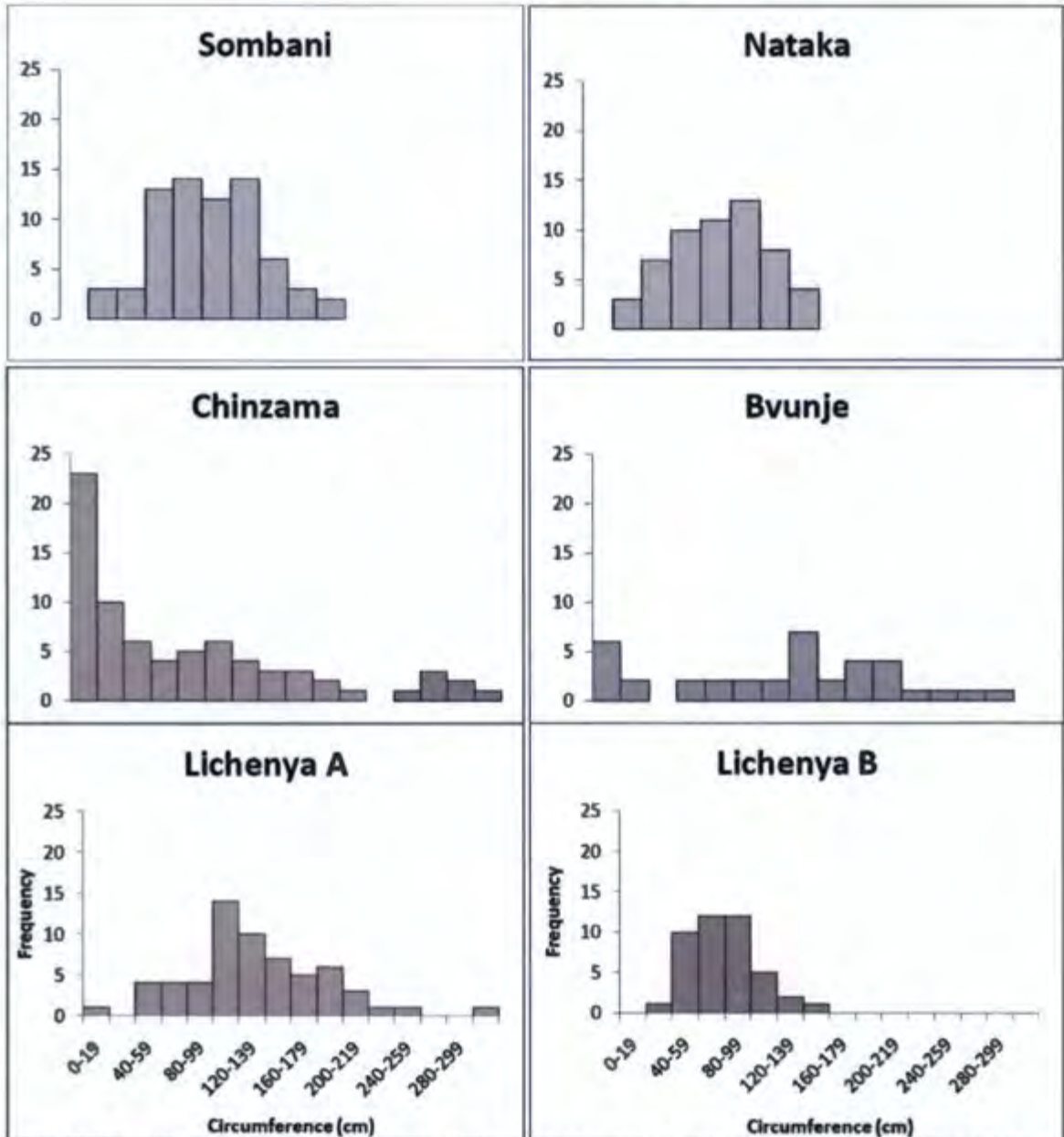


Figure 1.1 Size class distribution of six populations(Sombani, Nataka, Bvunje, Chinzama, Bvunje ,Lichenya A and Lichenya B) of Mulanje Cedar, Mount Mulanje, Malawi.

Overall, Chinzama show the highest numbers for this recruitment size class. Bvunje shows a more or less flat distribution, with overall low numbers in all size classes. The lack of trend in Bvunje's distribution pattern is despite measurements of harvested stumps being included in an attempt to depict what the population may have looked like without anthropogenic interference. It does however show some recruitment in the 0-19cm size class. Lichenya A and Lichenya B both show distributions that are approaching normal, though not as strongly as Sombani and Nataka, Lichenya A is skewed slightly to the right with minimal juveniles in smallest class.

Stable carbon isotope ratios

There is strong correlation between circumference and height of trees (figure 2.1) and therefore circumference is interchangeable with height as a measure of tree size. However, tree height relates more directly to the hypothesis that with increasing height and access to light that there will be increasing $\delta^{13}\text{C}$ values. We have therefore used tree height in all analysis except in figure 2.5 where only circumference values were available for non-*W. whyteii* trees and circumference of *W.whyteii* was also used as a means of comparison.

There is a strong positive correlation between height and $\delta^{13}\text{C}$ values ($R^2 = 0.6702$, figure 2.2) for *W.whyteii*. When these trees are grouped into those which were growing in the shade and those which were exposed to shade a new relationship appears. For those growing in the shade there is a strong positive correlation ($R^2 = 0.6074$) between height and $\delta^{13}\text{C}$ values figure 2.3). However, figure 2.4 shows there is only a poor ($R^2 = 0.4423$) correlation between height and $\delta^{13}\text{C}$ values for those trees found growing in the sunlight.

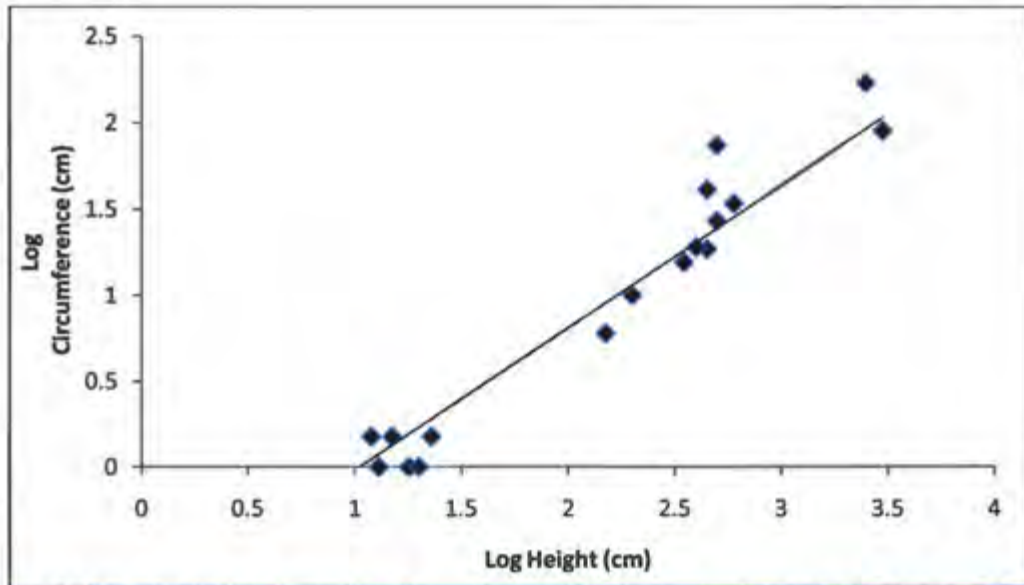


Figure 2.1 log of height plotted against log of circumference of *Widdringtonia whytei*, taken from Lichenya A and Lichenya B populations, Mnt Mulanje, Malawi. The graph shows a correlation coefficient $R^2 = 0.9518$.

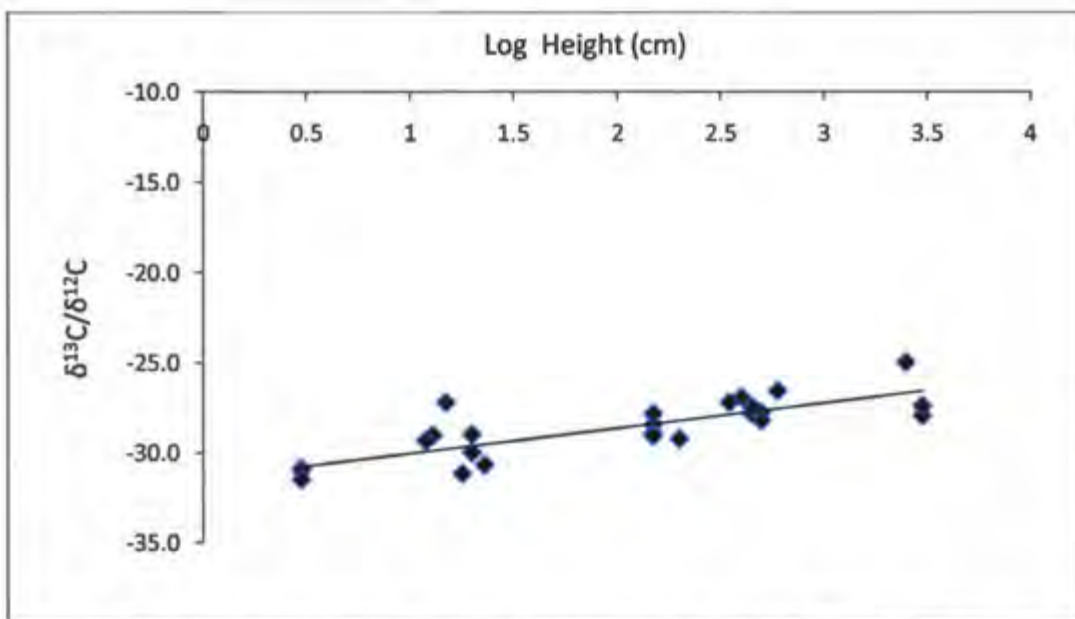


Figure 2.2 Correlation between Log of tree height plotted against the ratio of $\delta^{13}\text{C}$ to C^{12} for leaves from the Bvunje, Lechenya A and B sites, Mount Mulanje, Malawi, with $R^2 = 0.6702$.

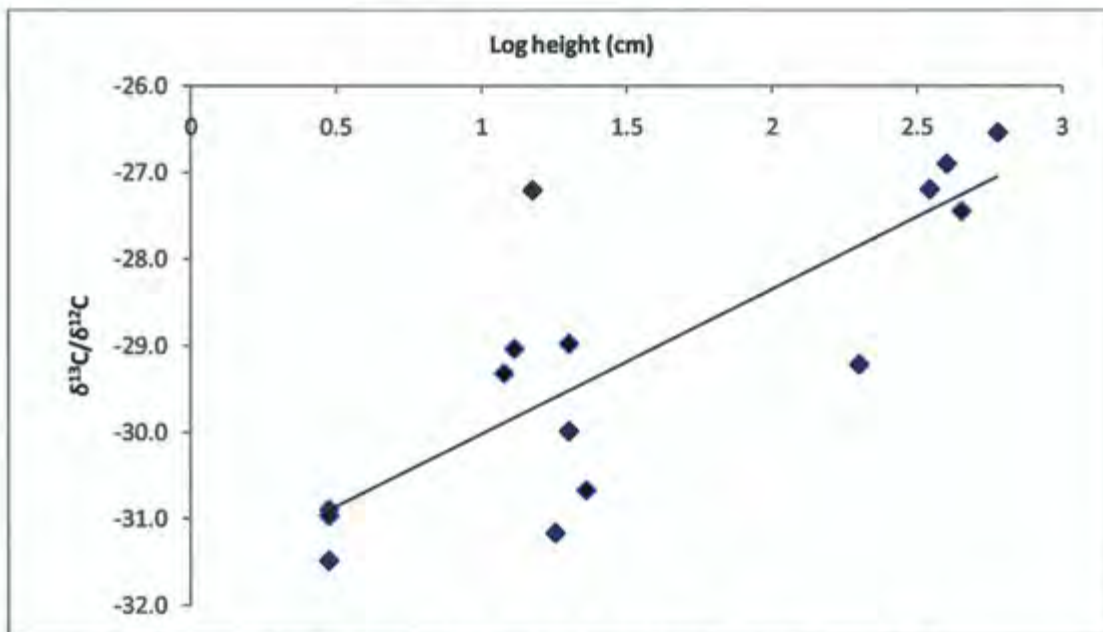


Figure 2.3 Carbon isotope ratio plotted against log of height for *Widdringtonia whytei* growing in the shade in the Bvunjje, Lechenya A and B sites, Mount Mulanje, Malawi. $R^2 = 0.6704$.

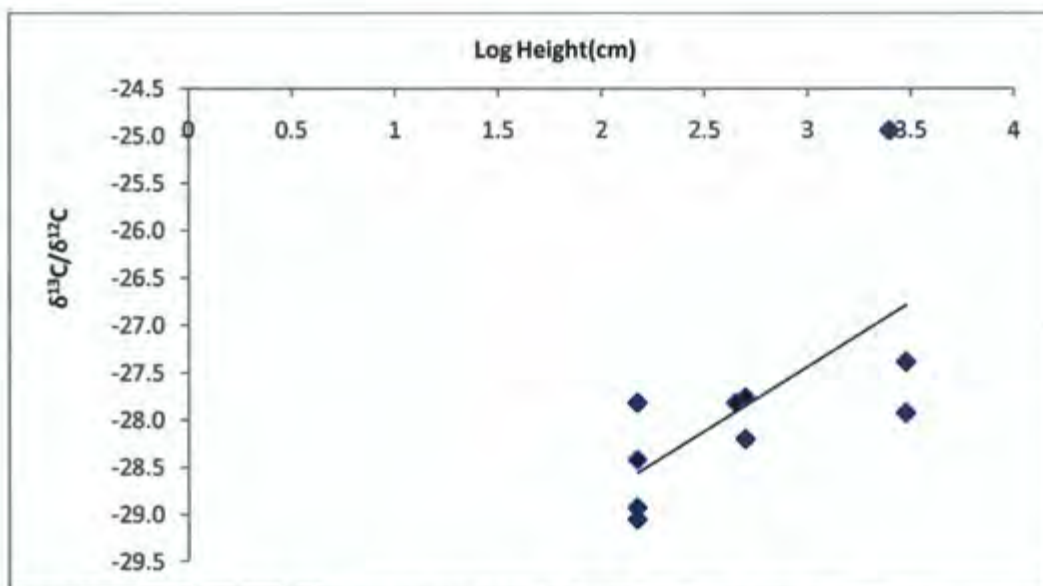


Figure 2.4 carbon isotope ratio plotted against log of height for *Widdringtonia whytei* trees growing in the shade collected from Lechenya A and Lechnya B sites, Mount Mulanje, Malawi, with $R^2 = 0.4423$.

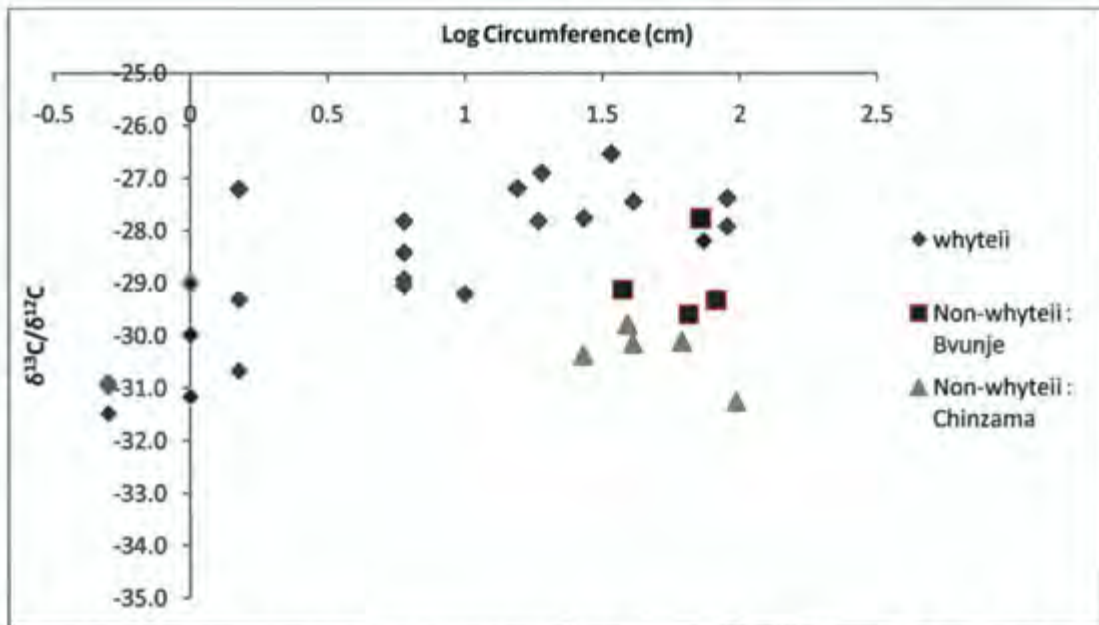


Figure 2.5 Carbon isotope ratios plotted against log of circumference for *Widdringtonia whyteii*, and Non-*Widdringtonia whyteii* trees which cohabited forests in Chinzama and Bvunje populations. $R^2 = 0.6823$ for *Widdringtonia whyteii*, $R^2 = 0.0242$ for Bvunje (Non-*Widdringtonia whyteii*) and $R^2 = 0.4374$ for Chinzama (Non-*Widdringtonia whyteii*).

There is a strong positive relationship between the circumference of *Widdringtonia whyteii* and carbon isotope values, relative to the poor correlations for the non-*whyteii* trees (Figure 2.5). Those trees from the Chinzama site show a weak negative correlation, while those from Bvunje show a weak positive correlation. Both groups of non-*W.whyteii* trees group in a site specific manner, with the Bvunje site showing less negative values than the Chinzama site.

There was a significant difference between the $\delta^{13}\text{C}$ values for the inner and the outer sections of wood from the Bvunje population ($p = 0.005062$). There was no significant difference ($p=0.066317$) in $\delta^{13}\text{C}$ values between the inner and outer wood in the Sombani site. There was no significant difference ($p = 0.909722$) in $\delta^{13}\text{C}$ values in the outer wood between the two populations while there was a very significant difference ($p = 0.000183$) between the inner wood of the two sites.

Discussion

My data shows that four of the six populations appear to have distributions which are approaching a normal curve, suggesting that the trees arose as single cohort (Rubin *et al* 2006). In contrast, Chinzama shows a distribution which is largely composed of juveniles with few mature adults, indicative of a healthy population which recruits slowly over time, producing more juveniles than are expected to survive to adulthood (Rubin *et al* 2006). This trend is site-specific as it only appears in Chinzama.

The data from Bvunje is unclear and does not show a particular trend in tree growth. Although, it is a heavily poached site, tree stumps were measured as well as live trees, in order to show what the size class structure may have looked had there not been any anthropogenic impact. It is not known the historical extent of poaching in this site and how this may have affected the size class distribution over time, but one would expect poaching to remove only the largest trees and not to cause a flat distribution. As with Chinzama, this is a site-specific trend. Despite the ambiguities of Chinzama and Bvunje, the populations seem to indicate single cohort recruitment.

The carbon isotope values of the *Widdringtonia whytei* leaves are a good indicator of tree height (fig 2.3) particularly if the tree is not yet exposed to full sun (fig 2.4). This is in agreement with Berry *et al* (1997) who found a strong relationship between height and leaf $\delta^{13}\text{C}$ value. The weak correlation shown in figure 2.4 could be due to sun being the predominant influence on trees when they are exposed to little sunlight, but that as soon as sunlight is readily available to them than other factors affecting carbon isotope values, such as water availability (Farquhar *et al* 1989), become more important in influencing the carbon isotope ratio.

It is possible that some species/leaves photosynthesise rapidly when small patches of light, sunflecks, fall on the leaves. This changes the ratio of supply and demand of CO_2 and $\delta^{13}\text{C}$ values of the leaf. For this reason, there can also be differences in $\delta^{13}\text{C}$ values for leaves at a constant height, depending on their level of exposure to light (Pearcy and Pfitz 1991). This could account for variance in the strong but not fully explained correlation between height and $\delta^{13}\text{C}$ values (figure 2.2).

There is a weak correlation between circumference and $\delta^{13}\text{C}$ values for non-*W.whytei* trees (figure 2.5). This may be due to small sampling size or to several different species being included in the analysis. This could be due to the large amount of variability in $\delta^{13}\text{C}$ values between species (Chevilatt *et al* 2005). Therefore, I suggest future sampling should include bigger sample group from selected species only, instead of a variety in order, to account for this variability. The non-cedars seem to group according to their sites which is likely due to site-specific factors such as water availability (Warren *et al* 2005).

There is no significant difference between the carbon isotope ratios in the inner and outer wood of the Sombani site or between the outer wood of the two sites. This suggests that, having broken the canopy and being exposed to the light, the Mulanje cedars are experiencing the same conditions in both sites. Sombani population seems to be experiencing the same conditions now as it was during recruitment, suggesting that they did not recruit from beneath the canopy but instead grew up in full sun. However, there is a significant difference between the carbon isotope ratios of the inner and outer wood within the Bvunje site and the inner wood between Sombani and Bvunje sites. This suggests that recruitment conditions were different to those being experienced now. This would mean that the Bvunje population recruited in shade. This might also indicate that the Sombani and Bvunje sites recruited in different environments, and that the Mulanje Cedar does not have a preferred recruitment environment. Alternatively, other factors such as edaphic, atmospheric and meteorological factors may be altering carbon isotope ratios (Warren *et al* 2005).

It is possible that other environmental factors, such as water stress, might also account for this difference. It should also be noted that the sampling size ($n=10$) was fairly small and Berry *et al* (1997) noted significant variability between individuals of the same species.

Conclusion

The results obtained from both the size class data and the carbon isotope ratios do not give conclusive results as to the recruitment environment for the Mulanje cedar. However, the recurrence of normal distributions in several sites and the one skewed distribution suggests that the trees can recruit both as a single cohort and continually through time. The comparison of Sombani and Bvunje carbon isotope values also differ in terms of what

environments the seedling establish in, and this could mean that they are capable of doing so in both sunlight, exposed without being shaded by the canopy as well as in beneath the canopy in the shade. In conclusion, based on results of this study I cannot determine an exact recruitment environment for the Mulanje cedar, *Widdringtonia whytei*.

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Images:

Site Map was created using Google Earth 2010.